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Rapid anxiolytic activity of progesterone and pregnanolone in male rats

Claudia Gomez[a], Alfredo Saldivar-Gonzalez[a], Guillermo Delgado[b], Rodolfo Rodriguez[a,*]

[a]*Department of Pharmacology, Faculty of Medicine, National University of Mexico, P.O. Box 70-297, Mexico City 04510, Mexico*
[b]*Institute of Chemistry, National University of Mexico, P.O. Box 70-297, Mexico City 04510, Mexico*

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Abstract

The effect of different doses of progesterone (1.0, 3.0, 10.0, 30.0, and 100.0 mg/kg) and pregnanolone (1.0, 3.0, 10.0, and 30.0 mg/kg) upon burying defensive and elevated plus-maze (EPM) tests was investigated in adult male rats and compared with the effects of diazepam (0.25. 0.50, 1.0, and 2.0 mg/kg). All drugs were suspended in a 0.2% methylcellulose solution and administered intraperitoneally 30 min prior to testing. Progesterone and pregnanolone were found to produce anxiolytic-like effects similar to those of diazepam. Thus, at certain doses, both drugs significantly increased the latency for burying and decreased the cumulative burying behavior, without modifying the number of shocks, and increased the time spent in the open arms of the maze, without affecting the spontaneous locomotor activity. These data clearly demonstrate that the defensive burying paradigm is useful to detect the anxiolytic-like properties of pregnanolone. An important finding was that progesterone produces significant behavioral effects 30 min after its administration. This finding suggests a rapid bioconversion of progesterone to its active ring-A reduced metabolites; however, the possibility remains that rapid behavioral effects of progesterone are due to a direct interaction with specific steroid receptors located on the plasma membrane, independently from the γ-aminobutyric acid$_A$ (GABA$_A$) receptor complex modulation.

Keywords: Anxiety; Defensive burying behavior; Elevated plus-maze; Male rats; Progesterone; Diazepam; Pregnanolone

1. Introduction

Several lines of evidence indicate that progesterone is a steroid that may not only act on the genome to regulate gene expression but also has a very rapid, nongenomic, membrane-mediated mechanism of action. This conclusion is based on data from several model systems. For example, progesterone has been shown to: induce maturation of *Xenopus laevis* oocytes through interactions with the cell membrane (Baulieu, 1983); potentiate γ-aminobutyric acid$_A$ (GABA)-evoked inhibition of rat cerebellar Purkinje cells (Smith et al., 1987a,b); decrease absolute firing rates of Purkinje cells during stationary and locomotor phases in freely moving rats (Smith et al., 1989); suppress norepinephrine induction of cAMP (Petitti and Etgen, 1992); inhibit the binding of muscarinic agonists to hypothalamic and pituitary membranes (Klangkalya and Chan, 1988); bind to σ-receptors in the guinea pig brain (Su et al., 1988); cause redistribution of oxytocin receptors in the hypothalamus (Schumacher et al., 1990); stimulate calcium influx and the acrosome reaction in human sperm (Osman et al., 1989); and decrease responses of the guinea-pig ileum to electrical stimulation (Rodriguez et al., 1996). These effects occur with a latency of seconds to a couple of minutes. Furthermore, specific binding sites for progesterone have been found to be associated with synaptic plasma membrane (Towle and Sze, 1983).

Progesterone has also been shown to reduce anxiety (Rodriguez-Sierra et al., 1984; Picazo and Fernández-Guasti, 1995). The ability of this and other neurosteroids to decrease anxiety-induced behavior has been demonstrated in several test procedures (Wieland et al., 1991; Pick et al., 1996; Brot et al., 1997), including the elevated plus-maze (EPM) (Bitran et al., 1991; Wieland et al., 1995) and the defensive burying paradigm (Picazo and Fernández-Guasti, 1995), and there is evidence that the anxiolytic effect of progesterone is mediated by its ring-A reduced metabolites, allopregnanolone (3α-OH-5α-pregnan-20-one) and pregnanolone (3α-OH-5β-pregnan-20-one) (Mok and Krieger, 1990; Bitran et al., 1995). It is now widely accepted that these neurosteroids exert many of their central effects through positive allosteric modulation of the GABA$_A$

* Corresponding author. Tel.: +52-55-623-2162; fax: +52-55-616-1489.

E-mail address: rodcar@servidor.unam.mx (R. Rodriguez).

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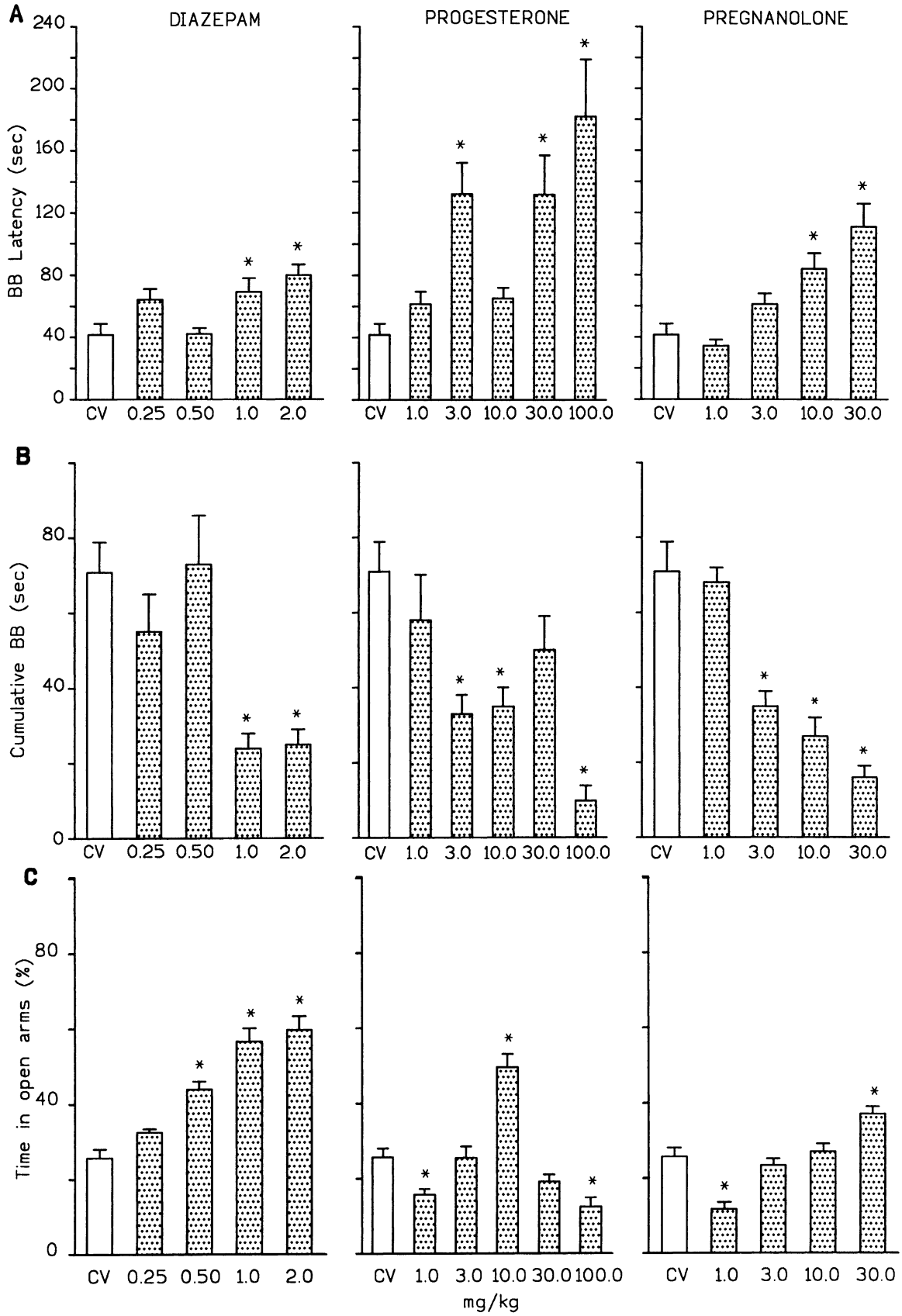
A
DIAZEPAM
PROGESTERONE
PREGNANOLONE
BB Latency (sec)
240
200
160
120
80
40
0
CV 0.25 0.50 1.0 2.0
CV 1.0 3.0 10.0 30.0 100.0
CV 1.0 3.0 10.0 30.0
B
Cumulative BB (sec)
80
40
0
C
Time in open arms (%)
80
40
0
mg/kg

receptor complex (Majewska, 1992; Lambert et al., 1995). For example, at nanomolar to low micromolar concentrations, these steroids potentiate GABA responses (Lambert et al., 1987), stimulate [^{3}H]muscinol and [^{3}H]flunitrazepam binding to the $GABA_A$ receptor complex (Majewska et al., 1986; Morrow et al., 1987), and displace [^{35}S]*t*-butylbicyclophosphorothionate from its binding site (Majewska et al., 1986). Noteworthy is that the ring-A reduced metabolites are exceptionally potent, positive modulators of $GABA_A$ receptors (Harrison et al., 1987), whereas progesterone itself is far less effective.

Consistent with experimental data indicating that the behavioral effects observed after progesterone administration are due to its biotransformation to pregnanolone and allopregnanolone, which subsequently augment $GABA_A$ receptor-mediated functions, it is widely accepted that such effects occur with a time-lag of several hours. Although no information on the time-course of the anxiolytic effects of progesterone is available, in most experimental studies this hormone is given 4 h before testing to allow adequate absorption, distribution, and biotransformation to its neuroactive metabolites (Bitran et al., 1993; Picazo and Fernández-Guasti, 1995). However, as mentioned before, several studies have shown that some progesterone actions can be extremely rapid and, therefore, unlikely to be exerted via its bioconversion to active metabolites.

We chose to study the anxiolytic effects of progesterone and pregnanolone because our initial screening studies on a large group of steroids, using the procedure described by Irwin (1968), revealed that the pharmacological profile of progesterone and pregnanolone is very similar to that of benzodiazepines and their behavioral changes are clearly established 30 min after intraperitoneal administration.

In this study, we evaluated the anxiolytic properties of progesterone in noncastrated adult male rats using two anxiety models. Results were compared with diazepam and pregnanolone, the 5β-reduced pregnane metabolite of progesterone, which has been shown to be as potent as allopregnanolone in the allosteric modulation of $GABA_A$ receptors (Gee and Lan, 1991) and to produce stronger anxiolytic effects than those of the 5α-isomer (Wieland et al., 1995), but reported to be inactive in the defensive burying test (Picazo and Fernández-Guasti, 1995), a very well-established animal model of anxiety (Treit et al., 1981).

2. General methods

2.1. Preliminary experiments

Male adult mice (CFW), 30–40 g, were used in preliminary experiments. Central nervous system effects of several progestins and diazepam were first evaluated as described by Irwin (1968). Graded doses (1–300 mg/kg) of each drug were given intraperitoneally to groups of mice (two animals for each dose), and the animals were observed continuously over a period of 6 h and then every 6 h up to 24 h. Diazepam, progesterone, and pregnanolone produced passivity and reduced spontaneous motor activity, muscular tone, muscular force, escape responses, and motor coordination. These effects tended to be dose-dependent and were clearly established 30 min after drug injection, decreased at 6 h, and disappeared 24 h after drug administration. Based on these observations, a 30-min treatment interval was chosen to perform the following series of experiments.

2.2. Animals

Adult Wistar male rats weighing 250–300 g were used in this study. All animals were housed six per cage (cage size: 55×35×20 cm, acrylic) for at least 1 week prior to testing. Animals were maintained in a controlled temperature (21±2 °C) and humidity (60±2%) environment under an inverted 12-h light–dark cycle (lights on at 10:00 p.m. and off at 10:00 a.m.). Seventy-two hours before the experiments were started, rats were moved to individual home cages (27×16×23 cm, acrylic). All tests were done 2 h after onset of the dark phase in a red dim chamber contiguous to the housing room. Food (Purina Rat Chow) and drinking water were freely available, except during testing. The research described in this report adhered to the National Health Ministry guidelines for the use of laboratory animals.

2.3. Behavioral procedures

2.3.1. Experiment 1: Effect of progesterone, pregnanolone, and diazepam on defensive burying behavior (DBB)

The DBB was assessed essentially as described by Treit et al. (1981). Briefly, the test apparatus was a 27×16×23-cm acrylic cage with 5 cm of bedding material (sawdust) spread evenly over the floor of the cage. This cage was provided with a shock prod (7.0×0.5×0.5 cm) emerging from the back wall and 2 cm above the bedding material, which delivered a low nonpainful electric shock (0.3 mA) each time the rat touched it. The electrical source consisted of a constant current shocker (Grass Medical Instruments, Model 54JR, Quincy, MA). At the beginning of the test, each rat was placed in the center of the cage and its behavior recorded during a 10-min period. The stereotyped movements of the forepaw aimed at covering the prod with sawdust were identified as DBB. We recorded the following variables in each rat: (a) latency to show burying behavior, i.e., the time in seconds elapsed between the first shock and the burying behavior; (b) the cumulative burying behavior,

Fig. 1. Effect of various doses of diazepam, progesterone, and pregnanolone on burying behavior latency (panel A), cumulative burying behavior (panel B), and time spent in open arms of the EPM (panel C) in intact male rats. Animals were injected intraperitoneally with vehicle (open bars) or drugs under study (pointed bars) 30 min before testing. Bars indicate mean ± S.E.M of 7–15 animals per dose. *Significant differences vs. vehicle control (Dunnett's test).

i.e., the time in seconds that each rat spent burying the prod during the ensuing 10-min test period; (c) the number of shocks received during the 10-min test period; and (d) the height of the sawdust pile at the end of the 10-min test. In this test, 164 rats were randomly assigned to different treatment conditions: control–control (CC); control–saline (CS); control–vehicle (VE); drug-treated animals with either progesterone (1.0, 3.0, 10.0, 30.0, and 100.0 mg/kg), pregnanolone (1.0, 3.0, 10.0, 30.0 mg/kg), or diazepam (0.25, 0.50, 1.0, and 2.0 mg/kg), and tested in counterbalanced order (one rat of each group everyday and tested in randomly allocated order) for DBB. Subjects showing freezing behavior at the beginning of the test were rejected.

2.3.2. Experiment 2: Effect of progesterone, pregnanolone, and diazepam on the EPM behavior

This test was done essentially as described by Pellow and File (1986). The apparatus consisted of two open arms (50×10 cm) and two enclosed arms (50×10×10 cm), arranged in such a manner that the two arms of each type were opposite to each other. The maze was elevated 50 cm above the floor. Incandescent 200-W light bulbs were placed above each open arm to produce a strong contrast between the open arms and the enclosed arms. At the beginning of each trial, which lasted 5 min, each rat was placed at the center of the plus-maze facing an open arm. We recorded the total number of arm entries (open+closed), the number of entries to the open arms, and the time spent in the open-arms section. All four paws had to be inside an arm for the dependent variable to be measured. The time spent in the center of the maze was not counted, so the total time spent in the open arms and the enclosed arms may not be equal to 5 min. The box was thoroughly cleaned with damp and dry towels after each test. For this experiment, 129 rats were randomly divided and tested for EPM under the following experimental conditions: CS, VE, and drug-treated animals. Steroids and diazepam doses were as in the first experiment.

2.3.3. Experiment 3: Effect of progesterone, pregnanolone, and diazepam on locomotor activity

Since behavioral alterations could just be a manifestation of changes in locomotion, we evaluated the effect of the two steroids and diazepam on locomotor activity of rats. In this group of experiments, locomotor activity was recorded in a square box (1×1 m), surrounded by a 30-cm wall. The bottom of the device was marked with 20×20 cm squares. Each rat was placed in the center of the device and the number of crossings from one square to another was recorded over a 5-min period. The box was thoroughly cleaned with damp and dry towels after each test. In this group of experiments, 98 rats were randomly divided and tested in counterbalanced order for spontaneous ambulatory behavior. Locomotor activity of each rat was recorded over a 5-min period. Doses of steroids and diazepam were as in the previous experiments.

2.4. Drugs

Progesterone and pregnanolone (3α-OH-5β-pregnan-20-one) were purchased from Sigma, Saint Louis, MO; Silanes Laboratories, Mexico, donated diazepam. Compounds were always suspended in a 0.2% methylcellulose solution, prepared fresh on test days, and administered intraperitoneally 30 min prior to testing (0.2 ml/100 g of body weight); all doses are expressed in terms of the salts.

2.5. Statistics

The parametric behavioral data (latency for burying, cumulative burying behavior, time spent in open arms, height of the sawdust) were initially analyzed through a one-way analysis of variance (ANOVA). Following significant ANOVA, Dunnett's *t* test was used to compare treatment groups with vehicle control. Differences in the number of shocks received and open-arm entries were calculated by Kruskal–Wallis ANOVA. Differences were considered statistically significant at $P < .05$.

3. Results

3.1. Experiment 1: Effect of progesterone, pregnanolone, and diazepam on DBB

Fig. 1 compares the burying behavior latency (panel A) and the cumulative burying behavior (panel B) 30-min after the intraperitoneal injection of drugs under study. Diazepam (1.0 and 2.0 mg/kg), progesterone (3.0, 10.0, and 100.0 mg/kg), and pregnanolone (3.0, 10.0, and 30.0 mg/kg) produced

Table 1
Effect of different doses of diazepam, progesterone, and pregnanolone on the behavior of male rats in the burying behavior test

Treatment[a]	Doses (mg/kg, ip)	*N*	Number of shocks (median)	Height of sawdust pile (cm)
Vehicle	–	13	3.0	5.15±0.37
Diazepam	0.25	8	4.0	4.10±0.49
	0.50	14	4.0	4.03±0.48
	1.00	7	4.5	3.00±0.37*
	2.00	10	3.0	2.40±0.54*
Progesterone	1.00	7	4.0	3.00±0.68
	3.00	8	4.5	2.56±0.70
	10.00	15	3.0	3.60±0.60
	30.00	8	4.0	4.00±0.70
	100.00	10	2.0*	1.40±0.77*
Pregnanolone	1.00	10	3.0	4.87±0.61
	3.00	9	4.0	3.42±0.64
	10.00	8	5.0	3.33±0.60
	30.00	8	5.0	2.75±1.09*

Data are mean±S.E.M.
[a] Given 30 min prior to testing.
* $P < .05$ against vehicle-treated animals.

a significant suppression of burying behavior; this effect was dose-related for pregnanolone. As seen in Fig. 1, the benzodiazepine and both steroids tended to increase the latency for defensive burying and the values produced by 3.0, 30.0, and 100.0 mg/kg of progesterone were substantially greater than those observed with pregnanolone and diazepam. In this test, 10 mg/kg of progesterone had no detectable effects on the latency for burying but significantly suppressed the cumulative burying behavior. As compared with diazepam, higher doses of each progestin were required to affect latency and cumulative burying behavior.

Except with the highest dose of progesterone (100.0 mg/kg), no significant changes in the number of electric shocks received were observed (Table 1). Diazepam (1.0 and 2.0 mg/kg), progesterone (100.0 mg/kg), and pregnanolone (30.0 mg/kg) significantly decreased the height of the bedding material (Table 1). Since no significant differences were found between animals receiving vehicle injection and saline-treated animals, and animals with no injection at all, these last two controls were not included in Fig. 1 and Table 1.

3.2. Experiment 2: Effect of progesterone, pregnanolone, and diazepam on the EPM test

The effects of diazepam, progesterone, and pregnanolone on rats assessed by the EPM test are shown in panel C of Fig. 1. Diazepam (0.25–2.0 mg/kg) produced a dose-dependent increase of the time spent in the open-arm sections of the maze. Pregnanolone produced a slight but significant biphasic effect; the lowest dose (1.0 mg/kg) decreased open-arms time, while the highest dose tested (30.0 mg/kg) increased it. A different pattern was observed with progesterone, which tended to be an inverted U. The lowest (1.0 mg/kg) and the highest doses (100.0 mg/kg) tested significantly decreased the time spent in open arms, whereas the intermediate dose (10 mg/kg) significantly increased this parameter. Drugs tested had no significant effect on the total number of arm entries (Table 2). All doses of pregnanolone increased the proportion of entries into the open arms. (Table 2). However, the effect was only significantly different from control at 10.0 mg/kg. Although some doses of progesterone produced an apparently greater proportion of entries into the open arms, the increase did not reach statistical significance. Neither dose of diazepam significantly affected this variable. Since no significant difference was found between animals receiving vehicle injection and saline-treated animals, this last control was not included in Fig. 1 and Table 2.

Table 2
Effect of different doses of diazepam, progesterone, and pregnanolone on the number of open-arm entries in the EPM

Treatment[a]	Doses (mg/kg, ip)	N	Total number of arm entries (open+closed)	Percent number of open-arm entries
Vehicle	–	8	12.1 ± 0.1	35.1
Diazepam	0.25	10	11.8 ± 0.3	39.1
	0.50	10	10.7 ± 0.2	34.0
	1.0	10	11.5 ± 0.7	35.7
	2.0	9	10.8 ± 0.5	3.40
Progesterone	1.0	9	12.8 ± 0.2	38.0
	3.0	9	11.5 ± 0.4	38.0
	10.0	8	9.8 ± 0.8	47.9
	30.0	7	10.5 ± 0.7	35.5
	100.0	7	8.8 ± 0.5	33.0
Pregnanolone	1.0	8	12.1 ± 0.4	39.6
	3.0	10	11.5 ± 0.3	53.7
	10.0	9	12.8 ± 0.5	58.6*
	30.0	8	11.8 ± 0.8	53.7

Data are mean ± S.E.M.
[a] Given 30 min before testing.
* $P < .05$ against vehicle-treated animals.

Table 3
Locomotor activity after intraperitoneal injection of diazepam, progesterone, and pregnanolone

Treatment[a]	N	Doses (mg/kg, ip)	Total number of counts/5 min
VE	7	–	85.8 ± 7.2
Diazepam	7	0.25	80.5 ± 7.1
	7	0.50	107.0 ± 6.6
	7	1.00	94.7 ± 8.3
	7	2.0	59.7 ± 3.7
Progesterone	7	1.0	86.0 ± 9.5
	7	10.0	87.1 ± 4.9
	7	100.0	82.2 ± 10.2
Pregnanolone	7	1.0	84.4 ± 9.0
	7	10.0	78.8 ± 5.1
	7	100.0	85.5 ± 4.4

Data are mean ± S.E.M.
[a] Given 30 min prior to testing.

3.3. Experiment 3: Effect of progesterone, pregnanolone, and diazepam on spontaneous ambulatory behavior

No changes in locomotion were found among tested groups (Table 3).

4. Discussion

The present results clearly show that: (a) the DBB is sensitive in a dose-dependent manner to the anxiolytic effects of pregnanolone, and (b) progesterone administered intraperitoneally 30 min before testing significantly affects the behavior of rats in both tests, defensive burying and EPM, although its behavioral profile was somewhat different from that of diazepam and pregnanolone.

In agreement with previous reports (Treit et al., 1981; Pellow and File, 1986; Rodgers and Johnson, 1998; Saldivar-Gonzalez et al., 2000), diazepam produced a behavioral profile consistent with anxiety reduction, i.e., it decreased cumulative burying behavior and the height of the displaced

bedding material, and increased the time spent in the open-arm sections of the maze, although at the tested doses it did not increase the proportion of the open-arm entries. In our experiments, diazepam also increased the burying behavior latency, reflecting a decreased reactivity (Treit et al., 1981). Pregnanolone follows a similar, although not identical, pattern of action. Its effects on cumulative burying behavior was clearly dose-dependent; it consistently increased the proportion of open-arm entries, but only the highest dose tested (30.0 mg/kg) increased the total time spent on open arms. Progesterone was found to produce the characteristic anxiolytic-type effects at doses of 10 mg/kg, whereas lower and higher doses decreased the time spent in the open arms of the maze and substantially increased burying behavior latency. In general, these results are consistent with other behavioral studies. Evidences indicating that progesterone and its 3α–5β derivative exert sedative–hypnotic and anxiolytic effects in female and male rodents have been found in several studies (Seyle, 1942; Bitran et al., 1991, 1995; Picazo and Fernández-Guasti, 1995; Wieland et al., 1995). The increase of burying defensive latency observed in the present study may be interpreted as a sedative–hypnotic effect instead of an anxiolytic one. However, the lack of effects with both steroids on locomotor activity and the observed decrease of the height of the displaced bedding material favor the idea of an anxiolytic-like effect of both neurosteroids. These results agree with those reported by Picazo and Fernández-Guasti (1995), stating that progesterone and some of its reduced metabolites diminish anxiety without modifying ambulatory behavior.

The results of the present study demonstrate that the DBB is sensitive to the anxiolytic-like effects of pregnanolone. We found that this steroid reduces burying behavior in a dose-dependent manner and consistently decreased the height of the bedding material. Other investigators have reported lack of sensitivity of this model to pregnanolone (Picazo and Fernández-Guasti, 1995). They administered doses of approximately 5–20 mg/kg, therefore, differences in dose levels do not explain this discrepancy. It is then possible that differences between this and previous reports might be related to the time of observation. Some authors follow the traditional pattern in this area of giving drugs (at fixed doses) 4 h before testing although pregnanolone is a rapid modulator of $GABA_A$ responses (Gee and Lan, 1991). In fact, pregnanolone has been shown by others to be a potent anxiolytic in the conflict test of Geller and Seifter (Wieland et al., 1995), the light–dark transition test (Wieland et al., 1995), and the EPM (Bitran et al., 1991; Wieland et al., 1995), when injected 10–30 min prior to behavioral tests. It is also possible that the inactivity observed by Picazo and Fernández-Guasti (1995) might be due to a lower bioavailability of pregnanolone when dissolved in dichloromethane and corn oil, and injected subcutaneously. In the current study, we used methylcellulose as vehicle, extended the dose-range tested, and administered progesterone intraperitoneally 30 min before testing.

The principal finding of this study is the rapid behavioral response to the administration of progesterone. This finding confirms the results of our screening study but apparently contradicts earlier work showing that this hormone exerts anxiolytic-like effects after its bioconversion into a number of ring-A reduced metabolites, including pregnanolone and allopregnanolone, which subsequently augments $GABA_A$ receptor-mediated function in a nongenomic manner. In fact, the anxiolytic effects of progesterone have been shown to be significantly correlated with the cortical levels of allopregnanolone, and its anxiolytic efficacy is also correlated with the maximal efficacy and potency of $GABA_A$-stimulated Cl^- transport (Bitran et al., 1993). Our finding that progesterone produces significant behavioral changes only 30 min after its intraperitoneal administration is not consistent with the notion that a lag period is necessary for the formation of the neuroactive metabolites from their parent hormone (Bitran et al., 1993, 1995; Picazo and Fernández-Guasti, 1995). However, it is quite consistent with the work of Smith et al. (1987a), who have demonstrated that progesterone, via either systemic administration or local application, significantly augments inhibitory responses of cerebellar Purkinje cells to GABA, and that this modulatory effect of the steroid occurs as early as 3–7 min after administration.

Since the time-course formation of ring-A reduced metabolites of progesterone and the onset and time-course of behavioral effects of progesterone are largely unknown, it is difficult to explain the biological significance of our finding. As mentioned before, one notable difference from the current finding and the previously reported anxiolytic-like effects of progesterone lies in the time between administration and behavioral testing. In some studies, testing is routinely done 4 h after drug administration, and information on onset and temporal course of the anxiolytic effect induced by progesterone is not usually obtained. In our study, testing time was based on the results of previous screening studies, in which animals were continuously observed during the first 6 h after drug administration, and then every 6 h up to 24 h. Besides, our results are consistent with the idea of a very rapid conversion of progesterone to $GABA_A$-active metabolites (Orchinik and McEwen, 1993). Since progesterone produced a somewhat different profile, it is possible that these atypical changes in rat behavior might be due to different biotransformation rates and bioavailability of ring-A reduced metabolites at the site of action. In favor of this view is the finding that its behavioral profile appears to be dose-related, i.e., the 10-mg/kg dose did not modify burying behavior latency, significantly reduced the cumulative burying behavior, and increased the time spent in the open arms of the maze, and elevated the percentage of open-arm entries, supporting the classical anxiolytic profile. Lower and higher doses did not follow this pattern and progesterone appears to be anxiogenic, since these doses significantly decrease the time spent in the open arms of the maze. The possibility that the peculiar behavioral profile of progesterone is due to simultaneous formation and participa-

tion of sulfated neurosteroids cannot be excluded since this type of metabolites negatively modulates $GABA_A$ receptors (Park-Chung et al., 1999) and can produce profound effects on behavior. In any case, this pattern of action deserves further research.

The finding that progesterone produces behavioral changes as early as 30 min after its administration supports the idea that this steroid is acting directly on plasma membrane receptors that are independent form $GABA_A$ receptors. However, 30 min might still be enough time for significant bioconversion (Dong et al., 2001). In addition, although there are no published behavioral data consistent with a rapid (<30 min) effect of progesterone via interaction with $GABA_A$ receptors, there is electrophysiological evidence for rapid effects of progesterone on $GABA_A$ receptors. Smith et al. (1987b) have shown that systemically administered progesterone increases the inhibitory response of Purkinje cells to GABA. This neuromodulatory effect is observed 5–15 min after progesterone injection and persists for 20–45 min poststeroid. That pregnanolone, and not the parent compound, is responsible for the effect is strongly suggested by findings that local application of the metabolite, but not progesterone, produced immediate (40–80 s) potentiating effects on GABA-mediated inhibition (Smith et al., 1987c). In contrast, local administration of progesterone produced significant enhancement of GABA inhibition only after constant exposure of the neuron to the steroid for 9 min, ample time for bioconversion of progesterone to the active metabolite. In addition, 4 MA, a drug that blocks the 5α-reductase enzyme and, thus, the formation of allopregnanolone, completely prevented the potentiating effect of progesterone on GABA inhibition (Smith, 1991). We think that these findings do not discard the possibility of a direct action of this hormone. In fact, the rapid behavioral effects of progesterone, as demonstrated in this study, may serve as an immediate short-term mechanism for steroid modulation of neural activity. Further experiments are currently under way to determine the temporal course of progesterone's anxiolytic action in the presence and in the absence of a progesterone receptor antagonist.

In summary, this study shows that the defensive burying test is an adequate paradigm to detect the anxiolytic-like properties of pregnanolone. In addition, it clearly demonstrated that, at some doses, progesterone possesses specific anxiolytic properties. Since the behavioral changes produced by progesterone take place within 30 min after administration, this finding suggests a very rapid conversion of progesterone to its ring-A reduced metabolites. However, the possibility that progesterone by itself, in the absence of further metabolism, is biologically active cannot be excluded.

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